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Outbreak of Hepatitis B Virus in Recent Arrivals to the Brazilian Amazon

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An outbreak of acute hepatitis cases in a small community took place 6 months after the community's arrival to the Brazilian Amazon. An epidemiological investigation was performed and included residents aged more than two years. Study subjects were interviewed and bled to test for hepatitis markers by enzyme immunoassays. Around 80% of the village population was surveyed. The overall prevalence of hepatitis B virus (HBV) markers was 75.1% (281/374). The surface antigen of HBV (HBsAg) and the IgM class antibody against hepatitis B core antigen (IgM anti-HBc) were present in 10.4% and 9.6%, respectively. Evidence of HBV–HDV (Delta virus) coinfection or hepatitis C infection was not found. IgM class antibody against hepatitis A virus was uncommon (3.7%). Follow-up evaluation 6 and 12 months later were carried out to identify new HBV infections. An incidence rate of 7.2 new infections per 100 exposed subjects per month was found. Average individual risk for HBV infection among susceptible inhabitants of the village between June 1995 and June 1996 can be estimated at 57.6%. The predominant HBsAg subtype found (ayw3) suggests that immigrants may have carried HBV from the original area. Time living in the study region was significantly associated with HBV markers in analysis for linear trend and logistic regression analysis. Environmentally related factors may have facilitated HBV transmission. ***J. Med. Virol. 56:4–9, 1998.*** © 1998 Wiley-Liss, Inc.

KEY WORDS: HBV subtype; incidence; epidemiology; emigration and immigration; Brazil

INTRODUCTION

Outbreaks of hepatitis B virus (HBV) are infrequent events. Reports have shown that HBV outbreaks take place inside risk groups, such as intravenous drug abusers and promiscuous subjects [Clee and Hunter, 1987], or in people exposed to the same contaminated source, such as blood products [Seef et al., 1987], acupuncture [Kent et al., 1988], or inadequately sterilized surgical instruments [Rimland et al., 1977; Polish et al., 1992; Harpaz et al., 1996]. In HBV hyperendemic areas, however, community-based outbreaks during which the mechanism of HBV transmission has not been clarified have sporadically occurred [Villarejos et al., 1972; Hadler et al., 1984; Hoffman et al., 1993]. In the Amazon region, this condition has been associated with Delta virus (HDV) superinfection in high-HBV prevalence populations [Hadler et al., 1984; Bensabath et al., 1987].

Toward the end of 1994, acute hepatitis cases started to be reported by public health authorities of Cotriguaçu, a county founded in 1976 by immigrants coming from South Brazil and located in the northwest part of Mato Grosso state, southern Brazilian Amazon. There are no data about viral hepatitis prevalence or attack rates in Cotriguaçu County before 1994. Most of the people affected lived in Nova Esperança, a small farmer community of new immigrants established in July 1993. An outbreak investigation was carried out in Nova Esperança in June 1995.

MATERIALS AND METHODS

The Nova Esperança village is located 60 Km outside of the main village of the county of Cotriguaçu. It was deforested in order to settle landless farmer families that had already been living under poor hygiene and crowded conditions in a region near the border between Brazil and Paraguay for more than two years. These families traveled 1500 Km northward to arrive in the settlement area. Most of them (55.8%) arrived to the study area in July 1993. Other immigrant families from the same original area arrived in the following months (30.3%). The rest (13.9%) of the community

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were immigrants from southern Brazilian states who were already living in Cotriguaçu county for more than two years. This low-income community, numbering approximately 450, lives basically by subsistence agriculture. Their houses were made of planks and each bedroom averages three persons. There is no sewage system and drinking water is supplied by wells. The only health worker based in the community uses disposable syringes. There is no data about hepatitis prevalence in the immigrants' original area. Few months after arriving in Nova Esperança, a large number of acute hepatitis cases broke out in this community.

The protocol used in the present study was approved by the Research Committee of the Federal University of Mato Grosso. Details of the objectives of the study were explained orally to all eligible participants and verbal informed consent was obtained prior to subject enrollment. A hepatitis case was defined as a self-reported jaundiced illness or as diagnosed by a physician. Blood for serological studies was collected from all consenting individuals over the age of two. An age of two years was established as the cutoff age because of the reluctance of the part of parents to have blood drawn from younger children. Sera were kept frozen at –20°C until tested at the Department of Virology, Fundação Oswaldo Cruz, Rio de Janeiro, Brazil. The samples were analyzed for HBV surface antigen and antibody (HBsAg and anti-HBs, respectively), total and IgM antibodies against HBV core antigen (anti-HBc and IgM anti-HBc, respectively), and IgM antibody against hepatitis A virus (IgM anti-HAV), using enzyme immunoassays (EIA) prepared by Instituto Oswaldo Cruz (FIOCRUZ). The reagent kits for the HBsAg and total anti-HBc EIA detection were recently evaluated in a blind comparison with a similar EIA kit as a gold standard showing high agreement rates [Souto et al., 1997]. The performance of the FIOCRUZ IgM anti-HBc test is periodically checked by comparison with similar commercial kits at the Department of Virology, Instituto Oswaldo Cruz. HBsAg subtyping was also performed by EIA using monoclonal antibodies prepared at Instituto Oswaldo Cruz [Niel et al., 1994]. Hepatitis C virus antibody testing (EIA, Abbott, Chicago, IL) was done by pooling five samples according to a protocol by Fernandez et al. [1995]. HBV e antigen (HBeAg) (EIA, Sorin, Saluggia, Italy) and test for IgG antibody against HDV (anti-HDV) (EIA, Abbott) were performed in HBsAg-positive sera. Jaundiced subjects without markers of recent HAV and HBV infection were tested for IgG class of antibody against hepatitis E (IgG anti-HEV) (EIA, Abbott). These tests were performed according to manufacturer's recommendations at Mato Grosso Federal University's Blood Bank, Cuiabá, Mato Grosso.

A subject was assumed to have been exposed to HBV if he or she had HBV markers (anti-HBc-isolated or anti-HBc with HBsAg or anti-HBs). Proportions were compared using the chi-square test with Yates' correction and Fisher's exact test. Crude odds ratios (OR) and relative risk (RR), with confidence interval, were used to determine the strength of association between variables. Chi-square for linear trend in proportions was calculated for stratified odds ratios. Models of logistic regression were constructed to adjust for confounders using the software EGRET (Cytel Software Corp., Cambridge, MA). Significance was determined at the 0.05 probability level in all analysis.

TABLE I. HBV Markers Distribution by Age Groups

Age	n	HBV markers-positive (%)[a]	HBsAg-positive (%)[b]	Anti-HBc IgM-positive (%)[c]
2–10	128	92 (71.9)	18 (14.1)	11 (8.6)
11–20	87	64 (73.6)	7 (8.0)	7 (8.0)
21–30	48	36 (75)	2 (4.2)	6 (12.5)
31–40	39	29 (74.4)	5 (12.8)	4 (10.3)
41–50	46	39 (84.8)	4 (8.7)	5 (10.9)
>51	26	21 (80.8)	3 (11.5)	3 (11.5)
Total	374	281 (75.1)	39 (10.4)	36 (9.6)

[a] $P = 0.54$ (χ^2 for linear trend).
[b] $P = 0.46$.
[c] $P = 0.92$.

RESULTS

In June 1995, 374 subjects from 104 households were interviewed and bled. Settlers living remote from the village or traveling during the study were not enrolled. More than 50% of the population were under 20 years old (Table I). Men and women were represented proportionately in the study sample. Reports of parenteral drug abuse, homosexual behavior, tattooing, and acupuncture were rare. Blood transfusions were reported by 23 subjects (6.2%). Only 10 (2.7%) subjects had been vaccinated against HBV previously. Skin lesions due to insect bites were found in 90.6% of the total sample, with some individuals exhibiting as many as 100 or more.

Two hundred and eighty-one (75.1%) people demonstrated HBV markers. There were 39 (10.4%) reactive for HBsAg and 36 (9.6%) for IgM anti-HBc (Table I). There were IgM anti-HBc-positive people in 28 different households. Twenty-one (53.8%) out of 39 HBsAg-positive were IgM anti-HBc-positive as well. HBeAg was tested in 37 of the 39 HBsAg-positive individuals and 24 (64.9%) of them were positive. Evidence of previous HBV infection (anti-HBc as the sole HBV marker or anti-HBc with anti-HBs) was present in 227 (60.7%). Ninety-three (24.9%) individuals had no HBV markers. Of the 36 (92%) HBsAg-positive individuals tested for anti-HDV, all were negative. In 19 serum samples among 39 HBsAg-positive, it was possible to subtype HBV: 14 (73.7%) were ayw3, 3 (15.8%) were adw2, and 2 (10.5%) were ayw2. There was no serum reactive for anti-HCV and 12 (3.7%) out of 322 sera tested for IgM anti-HAV were positive.

Seventy-one people (19%) self-reported previous hepatitis. Of these, 57 (80%) became ill after arriving in Nova Esperança; 54 (95%) of them showed evidence of having been exposed to HBV. The time distribution of

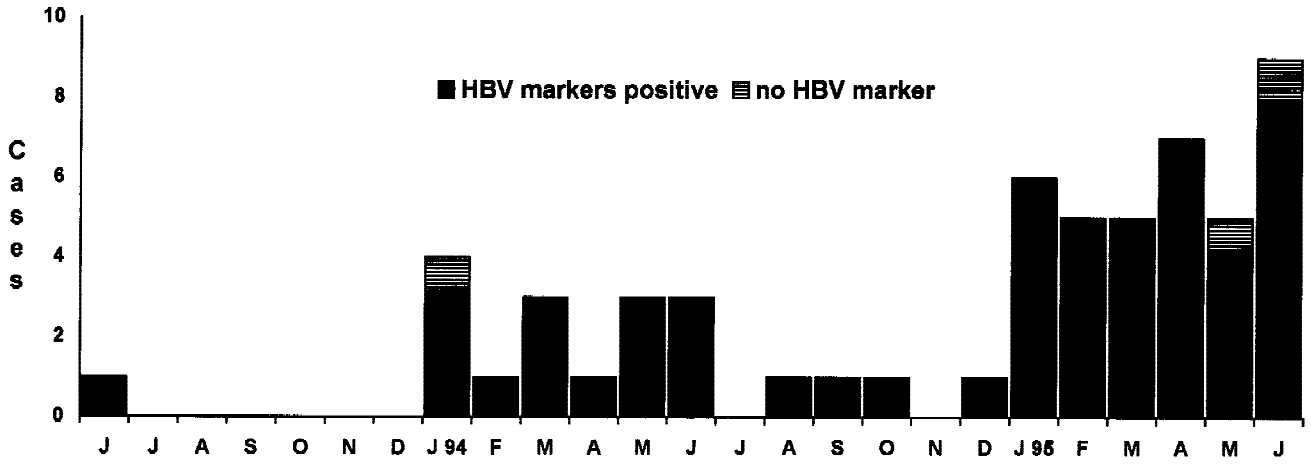

Fig. 1. Number of hepatitis cases occurring after people arriving in Nova Esperança by month of onset and presence of HBV markers (Anti-HBc alone or anti-HBc plus HbsAg or anti-HBs) as evaluated in June 1995.

possible cases of hepatitis is displayed in Figure 1. Thirty-four (91.9%) out of 37 reporting their illness in the last 6 months showed HBV markers. Ten (27%) of these had HBsAg and 12 (32.4%) had IgM anti-HBc. Nine were jaundiced, among whom seven (78%) had IgM anti-HBc and five (55%) were HBsAg-positive. The two jaundiced IgM anti-HBc-negative subjects also tested negative for IgM anti-HAV and IgG anti-HEV.

Two hundred and forty-nine (88.6%) out of the 281 subjects with HBV markers were from 95 (91.3%) of the 104 houses. Among these, there were 65 houses with two or more HBV-positive subjects, suggesting clustering of infection within families. No association was found between evidence of having been exposed to HBV and age, gender, ethnic group, skin lesions, sexual activity, dental treatment, hospitalization, blood transfusion, tattooing, or parenteral drug addiction. However, time living in the study area was significantly associated with either HBV markers or self-reported hepatitis using logistic regression models when adjusting for age, gender, and some HBV risk factors (Table II).

The notification of this outbreak investigation resulted in a vaccination campaign against HBV supported by the Brazilian ministry of health. This campaign was mainly directed at children under 15 years of age and was initiated several weeks after our visit. During this time, the serum specimens were tested. Hyperimmune hepatitis B immunoglobulin and vaccine against hepatitis A are rarely available in this part of the country.

In order to estimate the incidence of new HBV infections and carry out vaccination of susceptible people older than 15, a second visit to Nova Esperança was undertaken 6 months later. HBsAg, total anti-HBc, and IgM anti-HBc were retested in individuals with no HBV markers in June 1995. These individuals were asked for history of surgery, dental treatment, and whether they had left the study area between June and December 1995. Subjects that reacted for HBsAg in June 1995 were also retested. Since torrential rains and administrative problems resulted in poor follow-up, a third visit was made in June 1996.

Sixty-six (71%) of the 93 susceptible subjects were found and reevaluated in the follow-up visits (Table III). They were from 43 different households. Reevaluated subjects denied exposure to risk factors for HBV infection or trips to neighbouring counties since our first visit. Thirteen (19.7%) out of these 66 reported jaundice between evaluations and 37 (56%) had anti-HBc seroconversion. Seven (10.6%) of the overall retested individuals were also HBsAg-positive and five of these were IgM anti-HBc. Incidence density calculation demonstrated 7.2 new infections per 100 exposed persons per month (37 seroconversions per 516 person/months of observation).

The average individual risk for HBV infection among susceptible inhabitants of the village between June 1995 and June 1996 can be estimated by the formula: $R(t) = 1-\exp(-\mu t)$, where $R(t)$ is the average risk for development of disease among members of a cohort during a specified period; μ is the average value of the incidence rate during the period; and t is the period of estimated time [Kelsey et al., 1996]. In this way, the average individual risk for acquiring HBV infection in a year was 57.6%. However, among 66 reexamined subjects, 29 had received at least two doses of HBV vaccine, among whom 15 had anti-HBc seroconversion. These 29 vaccinated subjects presented an incidence density of 5.7 new infections per 100 exposed persons per month (15 seroconversions per 264 person/months of observation) and an average individual risk for HBV infection of 49.5%. On the other hand, the unvaccinated group had 8.7 per 100 exposed person per month (22 seroconversions per 252 person/months) and an average individual risk of 65%. There was no significant difference between the seroconversion rates of the two groups (RR = 0.9; CI 95% = 0.6, 2.2; $P = 0.7$).

On both follow-up visits, 26 (67%) out of 39 HBsAg-positive subjects were reevaluated. HBsAg remained

TABLE II. Crude Odds Ratio and After Adjustments for Age, Gender, and Other Variables[a]

Variable	Crude OR	Adjusted OR (95% CI) model 1[b]	*P*	Adjusted OR (95% CI) model 2[c]	*P*
Gender					
female (ref)	1	1		1	
male	1.4	0.7 (0.4; 1.3)	0.3	1.3 (0.8; 2.2)	0.3
Age (year)					
2–10 (ref)	1	1		1	
11–20	1.3	2.0 (0.8; 4.8)	0.1	1.1 (0.5; 2.2)	0.8
21–40	1.2	4.1 (1.6; 10.5)	<0.005	1.3 (0.5; 2.9)	0.6
>41	1.7	5.3 (2.0; 14.1)	<0.001	2.1 (0.8; 5.2)	0.1
Time in Amazon (months)					
1–12 (ref)	1	1		1	
13–24	3.6	2.1 (1.1; 4.4)	<0.05	3.5 (2.0; 6.0)	<0.001
>25	4.5	2.7 (1.1; 6.9)	<0.05	4.7 (1.9; 11.2)	<0.001
Surgery	1.2	0.8 (0.4; 1.7)	0.6	1.1 (0.5; 2.4)	0.7
Dental care	1.2	0.8 (0.4; 1.6)	0.5	0.9 (0.5; 1.7)	0.8
Transfusion	1.1	0.7 (0.2; 2.2)	0.5	0.9 (0.5; 2.6)	0.9

[a]Outcome: self-reported hepatitis (model 1) and HBV markers (model 2).
[b]Association between HBV markers and time living in the Amazon analyzed for linear trend; $\chi^2 = 21.5$; $P < 0.001$.
[c]Association between HBV markers and time living in the Amazon analyzed for linear trend; $\chi^2 = 5.8$; $P < 0.05$. HBV markers defined as anti-HBc alone or anti-HBc with HBsAg or anti-HBs.

TABLE III. Anti-HBc Seroconversion of 66 out of 93 Formerly Susceptible Individuals Specified by Age and HBV Vaccine Status

Age (year)	Vaccinated subjects[a] Anti-HBc status in follow-up			Unvaccinated subjects Anti-HBc status in follow-up		
	+	–	Total	+	–	Total
2–10	6 (50%)	6 (50%)	12	8 (57%)	6 (43%)	14
11–20	6 (54%)	5 (46%)	11	5 (71%)	2 (29%)	7
21–40	2 (50%)	2 (50%)	4	8 (73%)	3 (27%)	11
>41	1 (50%)	1 (50%)	2	1 (20%)	4 (80%)	5
Total	15 (51.7%)	14 (48.3%)	29	22 (59.4%)	15 (40.6%)	37

[a]Two doses of recombinant vaccine at least.

positive in nine (34.6%) subjects. One (7.7%) of the 13 positive IgM anti-HBc in June 1995 remained a HBsAg carrier. By contrast, eight (61.5%) out of the 13 IgM anti-HBc-negative subjects in June 1995 were still reactive for HBsAg ($P < 0.05$).

DISCUSSION

To our knowledge, this is the first report on HBV markers seroconversion incidence during an outbreak in the Amazon region. We found a high prevalence of subjects with evidence of HBV markers (75.1%), a high prevalence of HBsAg positivity (10.4%), and evidence for recent HBV infection in 9.6% of the overall sampled population. Although HBV markers prevalence before arriving to Nova Esperança was unknown, the IgM anti-HBc prevalence and the high rate of HBsAg-positive subjects with HBeAg (64.9%) suggest that the infection had recently been transmitted in the study area. The high proportions of IgM anti-HBc in the jaundiced subjects (78%), and in the possible hepatitis cases in the last 6 months (32.4%) are consistent with this hypothesis.

HAV infection was not frequent and there is no evidence of HEV infection among non-A, non-B jaundiced subjects. Although a lack of sensibility of the pool method performed to search anti-HCV antibody is possible, a significant role of HCV in this outbreak seems to be unlikely.

Usually, the pattern of HBV infection in hyperendemic areas is highly correlated with increasing age, with most people being infected before the age of 15 [Bensabath et al., 1987]. In the present study, the IgM anti-HBc distribution did not vary with age, suggesting that the majority of the population was exposed to HBV somewhat simultaneously after arriving in the study area. Analysis for trend and logistic models showing a positive correlation between HBV positivity and time living in area support this hypothesis.

In the reports on HBV outbreaks inside the rain forest, there is little information regarding how long people have lived in the study areas [Villarejos et al., 1972; Hadler et al., 1984; Hoffman et al., 1993]. Furthermore, incidence rates of HBV infection hardly appears. We found an impressive incidence rate of HBV seroconversion after a short follow-up period. It was high even in subjects receiving two doses of vaccine. Some of these individuals probably were already infected before vaccination.

Most (92.3%) of the HBsAg-positive, IgM anti-HBc-positive individuals cleared the antigen after 12 months of follow-up. On the other hand, most (61.5%) of the HBsAg-positive, IgM anti-HBc-negative subjects

still remained HBV carriers after 12 months. These latter subjects probably represent the pool of HBV chronic carriers that perpetuates the infection in this community.

The source of introduction of the HBV into this community remains speculative. There is no HDV marker among HBsAg-positive individuals and the predominant HBV subtype was ayw3, usually found in southern Brazil. In the Amazon, HBV–HDV coinfection is very common and the most frequent HBV subtype is adw4 [Hadler et al., 1984; Gaspar and Yoshida, 1987; Arboleda et al., 1995]. These findings suggest that HBV arrived with the immigrants. Nevertheless, close to 15% of the surveyed subjects already lived in the county before the village establishment. They may have introduced the HBV in the new community.

In general, the route of HBV transmission in tropical areas is not clear [Villarejos et al., 1972; Hadler et al., 1984; Hoffman et al., 1993]. In the present study, the involved routes and factors were also not identified. Sexual transmission could not explain the overall HBV spread in this study in view of the large number of recently infected children. Although an implicated parenteral route cannot be reject, there was no massive use of intramuscular injection that could have contributed to HBV spread in the previous 6 months. Crowding, low socioeconomic, and bad hygienic conditions occurring in developing countries may contribute to HBV transmission [Mitch et al., 1974; Dibisceglie et al., 1986; Feret et al., 1987]. In all probability, household contact facilitated clustering of HBV infection, thus preventing the finding of any association between HBV markers and the above conditions or classical risk factors through statistical analysis. However, other factors also seem to play a role in the HBV transmission in the study place, since crowding and poor hygiene were already present in the original area.

Our data suggest that HBV transmission in this community was accelerated after immigration to the study area. Hence, we suppose that an environmental factor may have taken place in this setting. Open-skin lesions caused by scabbies or insect bites may be implicated in the HBV spread in the tropics [Hadler et al., 1984; Chanteau et al., 1993; Casey et al., 1996]. In our study, bites of the blackflies *Simulium oyapockensis* and *S. pertinax* were a common cause of pruriginous and bleeding wounds that may have facilitated HBV spread through person-to-person contact. However, as these lesions were almost universally present, no statistical association was found between them and HBV markers.

In conclusion, this community suffered an outbreak of HBV infection with a high incidence rate after moving to the Amazon. Since no other risk factor associated with HBV markers was found, we suppose that environmental factors may have facilitated the HBV transmission in the study area. After this study, we have recommended to the Brazilian public health officials to start vaccination against HBV for all people intending to move toward the northern part of Mato Grosso.

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